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Pathogenesis of Malformations in a Rodent Model for Smith-Lemli-Opitz Syndrome

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The fact that Smith-Lemli-Opitz syndrome (SLOS), a syndrome comprising major malformations involving a number of organ systems, results from an abnormality in cholesterol biosynthesis, was discovered only recently. Utilizing a drug (BM 15.766) to inhibit the same step in cholesterol biosynthesis as is abnormal in those affected with SLOS, we have developed a rat model that presents with abnormalities observed as early as gestational day 12 that appear to be consistent with some of those subsequent malformations that comprise the human syndrome. Abnormalities of the brain and face include deficiency in the midline region of the upper face, narrowing of the forebrain hemispheres and of the cerebral aqueduct, and deficiency in the developing lower jaw. Associated pathogenesis, as observed on gestational day 11 in histological sections and with scanning electron microscopy, involves abnormal cell populations at the rim of the developing forebrain and in the alar plate of the lower midbrain and hindbrain. The affected cells appear abnormally rounded up, having apparently lost their normal cell contacts. The potential basis for the selective vulnerability of this cell population and the impact of its vulnerability relative to subsequent dysmorphogenesis is discussed. Am. J. Med. Genet. 68:328–337, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: cholesterol biosynthesis inhibitor; animal model

INTRODUCTION

Every cell of the body requires cholesterol for membrane structure and integrity and, in the case of certain cell types, for steroid hormone or vitamin D synthesis. It is remarkable, considering the biological importance of cholesterol, that to date very little research has focused on altered cholesterol biosynthesis/concentration as it may relate to the genesis of birth defects. However, the recent discovery that the biochemical basis for a human malformation syndrome, Smith-Lemli-Opitz syndrome (SLOS), appears to lie in altered cholesterol biosynthesis, has stimulated new research efforts in this regard.

The SLOS, first described in 1964 [Smith et al., 1964], is an autosomal recessive malformation and mental retardation syndrome [see Opitz et al., 1994 for bibliographic review]. The considerable clinical variation that exists in this syndrome has resulted in its subdivision, in some reports, as SLOS types I and II [Gorlin et al., 1990]. However, Opitz et al. [1987] have suggested that this distinction is arbitrary and based primarily on severity. Herein, types I and II will be considered together. The clinical variation that exists among individuals manifesting SLOS leads to diagnostic difficulties, as many of the anomalies noted in this syndrome are also common in other malformation syndromes. Common manifestations in SLOS are craniofacial anomalies including microcephaly, broad nasal bridge, anteverted nares, cleft palate, micrognathia, and abnormal ears; central nervous system (CNS) defects including frontal lobe hypoplasia and absent corpus callosum (representing a form of holoprosencephaly), ventricular dilatation, partial agenesis of the cerebellar vermis, and mental retardation; limb anomalies, including syndactyly and polydactyly and rarely oligodactyly; urogenital abnormalities including hypospadias, ambiguous genitalia and renal abnormalities; and heart defects including patent ductus arteriosus (PDA), ventricular septal defects (VSD), atrial septal defects (ASD), and tetralogy of Fallot. Additionally, intrauterine growth retardation, unilobular lungs, and Hirschsprung disease are noted.

Based on abnormal serum steroids noted in two SLOS children, approximately 10 years ago Chaslow et al. [1985] suggested that SLOS might be due to a defect

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in steroid metabolism. Subsequent biochemical analyses described a specific defect in cholesterol biosynthesis as the basis for SLOS [Irons et al., 1993; Tint et al., 1994]. This result was confirmed subsequently in 33 patients [Tint et al., 1995a]. The biosynthetic abnormality results in low plasma cholesterol concentrations (below the 5th centile for age and sex-matched control children) and abnormally high concentrations of 7-dehydrocholesterol (values several thousandfold higher than in controls). Other tissues in affected individuals have shown similar profiles, leading Tint et al. [1995b] to conclude that it is probably defective local tissue synthesis of cholesterol and not merely an abnormally reduced circulating plasma cholesterol level that is responsible for the SLOS malformations. A defect in the enzyme 3β-hydroxysterol Δ^7-reductase (7-dehydrocholesterol Δ^7-reductase) that reduces the C-7,8 double bond of 7-dehydrocholesterol (the penultimate sterol in the cholesterol biosynthetic pathway) appears to be the primary metabolic defect. The teratogenic significance of the resulting low cholesterol versus high 7-dehydrocholesterol levels remains to be elucidated. 7-dehydrocholesterol reductase has not yet been sequenced, and its genomic locus remains unknown. However, recent mapping studies have localized SLOS to the distal part of the long arm of chromosome 7 (7q32.1) [Alley et al., 1995].

In addition to inherent abnormalities in the cholesterol biosynthetic pathway, drugs have been developed (primarily with the goal of reducing atherosclerosis) that yield similar end points. In the early 1970s, the developmental/teratogenic effects of interference with cholesterol metabolism following treatment with hypocholesteremic agents, including AY9944 [trans-1,4bis (2-dichlorobenzyl aminoethyl) cyclohexane dihydrochloride] began to be explored. AY9944 administration results in reduced cholesterol and elevated 7-dehydrocholesterol concentrations, apparently affecting 7-dehydrocholesterol reductase (the same biosynthetic step that is abnormal in SLOS). With the realization that cholesterol is one of the major lipids (25% by weight) in myelin, many of the developmental studies for which this drug was utilized concentrated on suppression of cholesterol biosynthesis during myelination of peripheral nerves [Smith et al., 1970] and in the CNS [Suzuki and DePaul, 1971]. Although much of the current literature that attempts to link abnormal cholesterol biosynthesis to the defects in SLOS has focused on deficient myelination, it is clear that much of the spectrum of malformations notable in SLOS (e.g., brain malformations, cleft palate, heart defects, polydactyly, etc.) is representative of abnormality beginning very early in embryogenesis.

Studies by Roux and his colleagues have been most informative relative to the effects of abnormal cholesterol biosynthesis occurring during the period of embryogenesis. Initial investigations by this group involved the use of triparanol, a cholesterol synthesis inhibitor whose target is different from AY9944 [Roux, 1964]. It blocks the reduction of desmosterol to cholesterol, resulting in teratogenic effects comparable to those observed by these investigators in later, more extensive analyses using AY9944. In 1979, Roux et al. reported that administration of AY9944 to pregnant rats early in gestation was teratogenic. The most characteristic malformation observed was holoprosencephaly, the least severe manifestation of the holoprosencephaly spectrum being represented by pituitary hypoplasia. Additionally noted were edema, hydronephrosis, testicular ectopy, ocular anomalies, and hydrocephalus. Administration of the drug on gestational day (GD) 4 resulted in reduction in maternal cholesterol that reached its lowest levels by GD10. In subsequent studies, this group was able to show that in order to cause teratogenesis, drug exposure resulting in reduction of maternal cholesterol levels below 30 mg/dl was essential [Roux et al., 1980; Barbu et al., 1984, 1988]. Feeding a hypercholesterolemia-provoking diet or oral administration of cholesterol concurrent with AY9944 treatment was successful in correcting some of the induced malformations, especially holoprosencephaly [Roux et al., 1979]. Of special note is a 1990 publication from Roux's laboratory describing analysis of the effects of AY9944 on rat embryos grown in whole embryo culture [Repetto et al., 1990]. The results of this study showed that rat embryos cultured on GD10 from dams that had been treated with AY9944 on day 4 of pregnancy, when grown in normal serum for 48 hours, had the malformations characteristically seen in vivo. Thus, it was concluded that the teratogenic insult is initiated very early in gestation (i.e., before their time of culture initiation on GD10).

Other compounds that act in a similar manner to AY9944 have also been developed. One of these, BM 15.766, [Pill et al., 1985; Aufenanger et al., 1985] appears to act at the same level in the cholesterol biosynthetic pathway as AY9944, with oral administration over a number of days resulting in lowered cholesterol and elevated 7-dehydrocholesterol levels. Xu et al. [1995] utilized this compound in rats and reported that BM 15.766 is a potent inhibitor of 7 dehydrocholesterol reductase, and that it can reproduce the cholesterol biosynthetic abnormality that is present in SLOS.

The current study was designed to examine the teratogenicity of BM 15.766 in rats. Of particular interest, considering a void in the literature relative to pathogenesis associated with malformations resulting from cholesterol-lowering drugs, was examination of embryos at the time that abnormal developmental events appeared to be initiated. To our knowledge, there are no published reports other than an initial abstract from our laboratory [Dehart et al., 1995] and one from Roux et al. [1995] regarding the teratogenicity of BM 15.766.

MATERIALS AND METHODS

Pregnant Wistar rats (Charles River, Raleigh, NC) were received either on the day of mating or 1 day later. They were housed under constant temperature and light conditions and had free access to a standard commercial rodent chow (Agway, Syracuse NY). Pregnant females were administered, by gastric lavage on 4 consecutive mornings, doses of 300 mg/kg BM.15.766 suspended in sesame oil at a concentration of 60 mg/ml. The drug was administered on days 4–7 or 6–9 of pregnancy. Control animals were administered the vehicle utilizing the same dosing regimen as that for the treated groups.

Dams were killed by CO_2 overdose on gestational days 12 or 13 (GD 12 or GD 13). Uteri were then removed and notation was made of numbers of implantation sites and apparent early resorptions. In a balanced salt solution, embryos were dissected free of extraembryonic tissues and embryonic membranes, with the exception of the amnion. All specimens were examined under a dissecting microscope to determine presence of malformation. Following fixation in 2.5% glutaraldehyde in Sorenson's phosphate buffer, selected embryos were processed for scanning electron microscopy (SEM) as described by Sulik and Johnston [1982]. Briefly, following glutaraldehyde fixation for 24 hours, embryos were rinsed, then postfixed in 2% osmium tetroxide for 2 hours. After dehydration in a graded ethanol series, the specimens were critical-point dried, mounted on metal stubs and sputter-coated with gold palladium. Electron microscopy was performed on a JOEL microscope.

For analyses of pathogenesis, embryos were examined on GD11. Selected specimens were processed and viewed with the scanning electron microscope as described above. Others were fixed in Bouin solution for subsequent histological analyses. Following fixation, specimens were cleared in ethanol and dehydrated for plastic embedding (JB4; Polysciences, Warrington, PA). Specimens were sectioned at 4 μm on a Sorval microtome and stained with methylene blue, acid fuchsin. Potential drug-induced excessive cell death was assessed using Nile blue sulphate vital staining as described by Sulik et al. [1988]. For this procedure, live embryos were incubated for 30 min at 37°C in a dilute (1:50,000) solution of Nile blue sulphate in Ringer's solution. Embryos were examined immediately and photographed.

Prior to the evaluation of the teratogenic effects of BM15.766, the hypocholesterolemic effect of the drug was tested on retired breeder male rats. These rats were treated, as above, on 4 consecutive days. Each rat provided samples for two time points, with blood drawn from the tail vein for the first determination, and from the abdominal aorta at the time of sacrifice for the second. Blood was collected at 0, 12, 18, 24, 36, 48, 60, 72, 96, 120, and 144 hours following initial dosing. At least two samples were collected for each time point. Plasma was immediately recovered by centrifugation and cholesterol level was determined by spectrophotometry according to the method of Allain et al. [1974] using an enzymatic kit (Sigma, St. Louis, Mo). This routine assay involves the oxidation of cholesterol to cholesterol-4-en-3-one by cholesterol oxidase with concomitent release of hydrogen peroxide. The hydrogen peroxide reacts with a chromogen and the intensity of the color produced is proportional to the cholesterol concentration in the sample. Blood was also collected from the abdominal aorta of the treated and control pregnant rats immediately following the removal of the uteri and processed as above for determination of cholesterol content. Additionally, since the specificity of the enzymatic kit for cholesterol is not known and it is possible that the expected high concentration of 7-dehydrocholesterol in treated animals may cross-react with the enzymatic assay, capillary-column gas chromatography-mass spectrometry was used to identify cholesterol and 7-dehydrocholesterol content in the plasma of control and treated pregnant rats [Tint et al., 1994].

All statistical analyses were performed using SAS (version 6.08 for personal computer). Differences in blood sterol values between treated and control rats, and differences in mean resorptions, embryos and malformed embryos were tested for significance by Student's test. The differences between groups in frequency of resorptions and malformations were analyzed using Chi-square.

RESULTS

The plasma cholesterol profile in retired breeder rats following a dose of 300 mg/kg BM 15.766 on 4 successive days is shown in Figure 1. On the third day of treatment, plasma cholesterol dropped from a baseline value of approximately 70 mg/dl to values below 30 mg/dl and remained below this level through the 7th day following treatment initiation.

Table I summarizes the reproductive data collected on day 12 of pregnancy from rats treated with BM 15.766 either from days 4 to 7 (group 1) or days 6–9 (group 2) of pregnancy and their respective controls. Increased incidence of developmental toxicity resulted from maternal treatment initiated either on day 4 or 6. However, the differences in incidence of malformed embryos reached statistical significance only in group 1 (treatment on days 4 to 7). BM 15.766 treatment also increased the resorption rates twofold, but this effect was not statistically significant in either group.

Maternal plasma cholesterol levels, measured by an enzymatic kit, on days 11, 12, and 13 of pregnancy remained significantly decreased 3 to 5 days after the last day of dosing and these values were significantly different from those of the corresponding control groups (Table II). This is consistent with results of our pilot trial showing the long-lasting hypocholesterolemic effect of BM 15.766 in retired male breeders with reduced blood cholesterol levels at least 4 days following the last treatment day (Fig. 1). Additionally, as shown in Table III, analyses utilizing gas chromotography-mass spectrometry confirmed that drug administration resulted in decreased cholesterol concentration and elevation of 7-dehydrocholesterol content from undetectable levels in control samples to levels that constituted close to

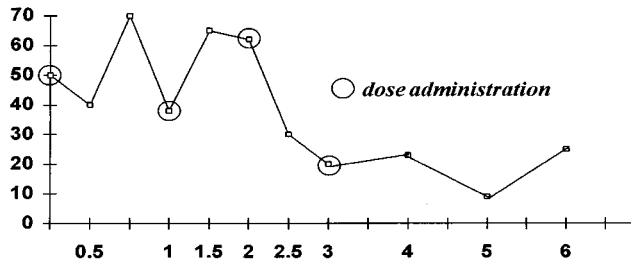

Fig. 1. Plasma cholesterol content (y axis) was determined at the time of initial dosing with 300 mg/kg BM 15.766, and at 12-hour intervals to day 3, then at 24-hour intervals to day 6, as indicated by the small boxes. Circled boxes indicate that drug was administered on days 0, 1, 2, and 3.

TABLE I. Developmental Toxicity of BM 15.766 Observed in GD 12–13 Mice Treated Between GD 4 to 7 (group 1), or From GD 6 to 9 (group 2)

	No. of litters	No. of implantations	Resorptions (%)	Live embryos (%)	Malformed embryos (%)
Group 1: treatment on GD 4–7					
Control	6[a]	77	5 (5.3)	72 (94.7)	1 (1.1)
Treated	8	115	12 (10.1)	103 (89.9)	60 (54.1)*
Group 2: treatment on GD 6–9					
Control	5	60	2 (3.5)	58 (96.5)	3 (6)
Treated	13	174	13 (7.2)	161 (92.8)	47 (33.2)

[a] Values represent total number of observations.
* Significantly different from corresponding control group at $P < 0.05$ by Chi^2 analysis.

50% of the combined values for cholesterol plus this metabolic precursor.

There was a significantly inverse correlation between maternal plasma cholesterol concentration measured on days 11–13 of pregnancy and the occurrence of embryonic malformation when the drug was administered between days 4 to 7, but this association could not be observed in animals treated between days 6 to 9 (Table II). Hence, although drug administration between days 6 to 9 still resulted in significantly decreased maternal plasma cholesterol, the teratogenic effect of the drug is stronger when treatment is initiated earlier (on days 4 to 7). These results are consistent with previous observations by Barbu et al. [1988] who showed that cholesterol supplementation of AY9944-treated dams starting on day 4 completely prevented the teratogenicity of AY9944, whereas cholesterol repletion from day 6 could not fully abolish the effect of the drug, suggesting that the earlier onset hypocholesterolemia is more damaging to embryonic development.

Initiation of treatment on GD 4 was, based on our preliminary plasma profile, expected to result in teratogenically low maternal plasma cholesterol levels (i.e., below 30 mg/dl) that persisted from GDs 7 to 12. The developmental stages present in rat embryos on the days corresponding to the period of low maternal cholesterol levels include preimplantation to early gastrulation stages (including the stages when the first few somite pairs are evident). As illustrated in Figure 2, the affected GD 12 embryos had malformations that were particularly evident in the upper midface (frontonasal prominence). Although various degrees of severity were noted, the defect consistently involved abnormally close approximation of the nasal pits. Of particular note, and as illustrated in Figure 2c, is dysmorphogenesis of Rathke's pouch, the tissue that will later form the anterior pituitary gland. Although, at this stage the upper portion of the face is abnormal in affected specimens, the pharyngeal arches, including the mandibular portion of the first arch which will form the lower jaw, appear normal. Abnormalities of the eyes were evident, presenting as a small optic vesicle/cup and failure of lens formation (Fig. 2f). Sagittal cuts through the brain illustrated narrowing of the cerebral aqueduct and diminished size of the cerebellar plate in affected embryos (Fig. 2g).

Initiation of treatment on GD 6 was expected to result in teratogenically low maternal plasma cholesterol levels that persisted from GDs 8 1/2 to 12. Developmental stages present in the rat at this time range from early postimplantation stages to those at which the neural tube completes its closure (approximately 29 somite pairs). As illustrated in Figure 3b and c, and as was common to the dysmorphogenetic effects noted following the earlier treatment regimen, in the affected GD 12 and 13 embryos, abnormality was notable in the developing forebrains, which were narrow relative to control embryos. Additionally, in some specimens there

TABLE II. Association Between Maternal Plasma Cholesterol Levels and Embryonic Malformations in Response to BM 15.766 Treatment

	No. of dams	Cholesterol (mg/dl)	Malformed embryos	Correlation coefficient
Group 1: treatment GD 4–7				
Control	6	66.4 ± 4.2[a]	0.17 ± 0.17	−0.82*
Treated	7	20.7 ± 2.9*	7.5 ± 2.6*	
Group 2: treatment GD 6–9				
Control	5	57.2 ± 3.4	0.6 ± 0.6	−0.32
Treated	10	19.3 ± 2.5*	3.9 ± 1.4	

[a] Values are means ± S.E.M.
* Indicates significant difference from corresponding control groups at $P < 0.05$ by Student's t test and by Pearson correlation test.

TABLE III. Plasma Cholesterol and 7-Dehydrocholesterol Levels in Pregnant Rat Dams Treated 4 Days With BM 17.566

	No. of dams	Cholesterol, mg/dl (% sterols)[a]	7-dehydrocholesterol (7-DHC, mg/dl) (% sterols)
Control	6	43.8 ± 5.7[b]	undetectable
Treated	15	6.8 ± 0.9*	6.13 ± 0.73
		(52.3 ± 3.1)	(47.7 ± 3.7)

[a] The word sterols is defined as the sum of cholesterol and 7-dehydrocholesterol.
[b] Values are means ± S.E.M.
* Indicate significant difference from corresponding control group at $P < 0.05$ by Student's t test.

was marked distension of the neural tube, with the hindbrain region being most affected (Fig. 3d–f). However, the lumen of the neural tube was enlarged at more caudal levels as well, as evidenced in Figure 3h in a cut made at the level of the upper limb bud. Additionally, the neuroepithelium of the developing spinal cord was abnormally reduced in thickness. Reduction in the size of the mandibular portion of the first pharyngeal arch, associated with distention of its vasculature, was also notable (Fig. 3b, c, i).

SEM and light microscopic (LM) analyses of embryos at earlier stages (GD 11) illustrated that selected cell populations in specimens from treated dams were abnormal (Fig. 4). The pattern of notable affect involved the rim of the anteriormost (forebrain) neural folds as well as the cells of the lower midbrain and hindbrain. Regarding the latter sites, those cells comprising the alar plate appeared to be most markedly affected. High magnification SEM and histological sections illustrated that the affected cells were rounded up, having lost their normal intercellular contacts. However, as confirmed by vital staining, excessive cell death was not a prominent manifestation.

DISCUSSION

Our results confirm and extend those of Roux [1964], Roux et al. [1979, 1980]; and Repetto et al. [1990] and, in concert with new data showing the teratogenesis resulting from altered cholesterol biosynthesis in humans (as observed in SLOS), highlight its significance for normal embryogenesis. As in humans with SLOS, in our model, the role of the low cholesterol levels versus other alterations resulting from interference with conversion of 7-dehydrocholesterol to cholesterol remains to be clarified. However, evidence points to the probability that low cholesterol levels alone act as a key player. Of particular note are previous studies by Roux et al. [1979] showing that cholesterol supplementation diminishes the teratogencity of AY 9944. Additionally, animal models recently produced using gene knockout technology and involving interference with cellular cholesterol transport and/or uptake, as opposed to reduced biosynthesis, either present with malformations and selectively affected cell populations similar to those in the biosynthesis inhibitor experiments [Homanics et al., 1995] or embryo lethality [Farese et al., 1995, 1996].

That the pathogenesis underlying the malformations in this model involves selective vulnerability of the cells at the rim of the anterior neural folds, including those of the lower midbrain and hindbrain alar plate, sheds light not only on our understanding of the SLOS phenotype, but also may provide clues to the cellular basis for similar malformations induced by other teratogenic agents. It is expected that insufficient cholesterol has a major effect on the cell membranes. Plasma membrane cholesterol content is known to influence membrane fluidity, which in turn affects many of the normal cellular functions. Deficiency in plasma membrane cholesterol may be a major factor underlying the abnormal cell rounding noted in some of the neuroepithelial cells in the affected embryos in this study. It is noteworthy that other agents that disorder the membrane by increasing its fluidity are also teratogenic. We have, for example, observed a remarkable similarity in the vulnerable cell populations and subsequent malformations resulting from ethanol exposure and in the model described herein and have documented a correlation between ethanol-induced increases in membrane fluidity and cellular toxicity at teratogenic concentrations [Sulik and Johnston, 1982; Sulik et al., 1984; Kotch and Sulik, 1992a,b; Chen et al., 1996]. Questions regarding the basis for the selective sensitivity of some cell populations following these teratogenic insults remain to be answered.

The malformations noted following both times of treatment initiation had some overlapping and some differing characteristics. Common to both exposure groups was varying degrees of facial and forebrain abnormality. These defects fall within the realm of holoprosencephaly. Previous studies in this laboratory that have involved acute ethanol exposure regimens in mice have shown that the sensitive period for the induction of this spectrum of forebrain deficiency is at gastrulation stages (GD 7–8 in mice). In the current study, both exposure times, i.e., GD 4–7 and 6–9, were expected, based on preliminary study of plasma profiles, to yield

Fig. 2. Scanning electron micrographs of affected (**b,c,e,g**) GD 12 rat embryos following maternal BM 15.766 treatment on GD 4–7 illustrate typical craniofacial dysmorphogenesis, as compared to comparably staged control specimens (**a,d,e**). Illustrated in a–c are frontal views; d and f are lateral views, and e and g are midsagittal cuts through the brain. Notable are the close proximity of the nasal pits (arrows in b and c) and narrowing of the forebrain region; the abnormal appearance of Rathke's pouch, the progenitor of the anterior pituitary gland (arrowheads in a,c, e,g); abnormal eye development as evidenced by the absence of a lens pit (compare arrow in d to the same region in f); narrowing of the cerebral aqueduct (arrows in e,g); and reduced size of the cerebellar plate (C in e and g). Bars = 100 μm (a,b) and 200 μm (c–g).

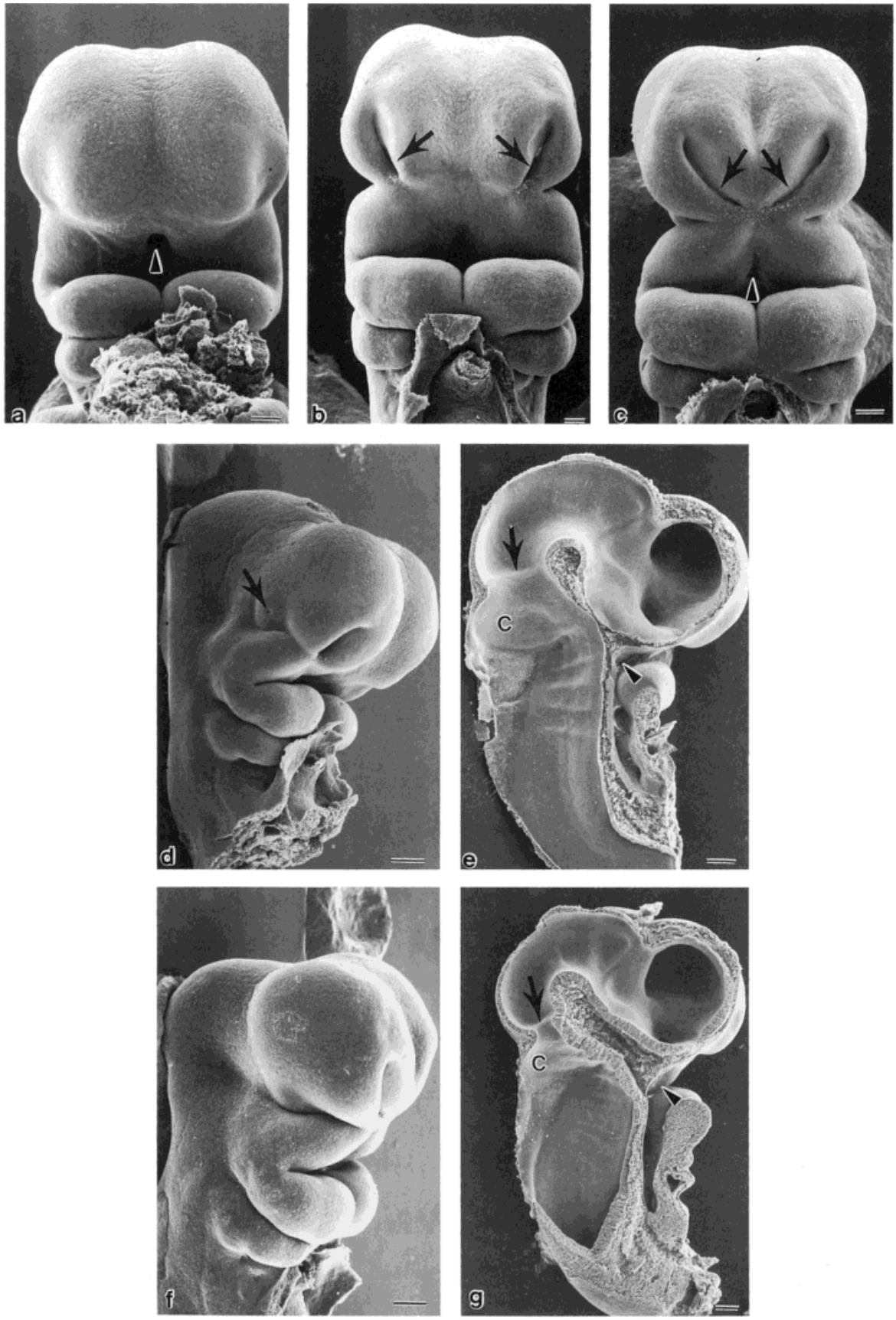

Fig. 2.

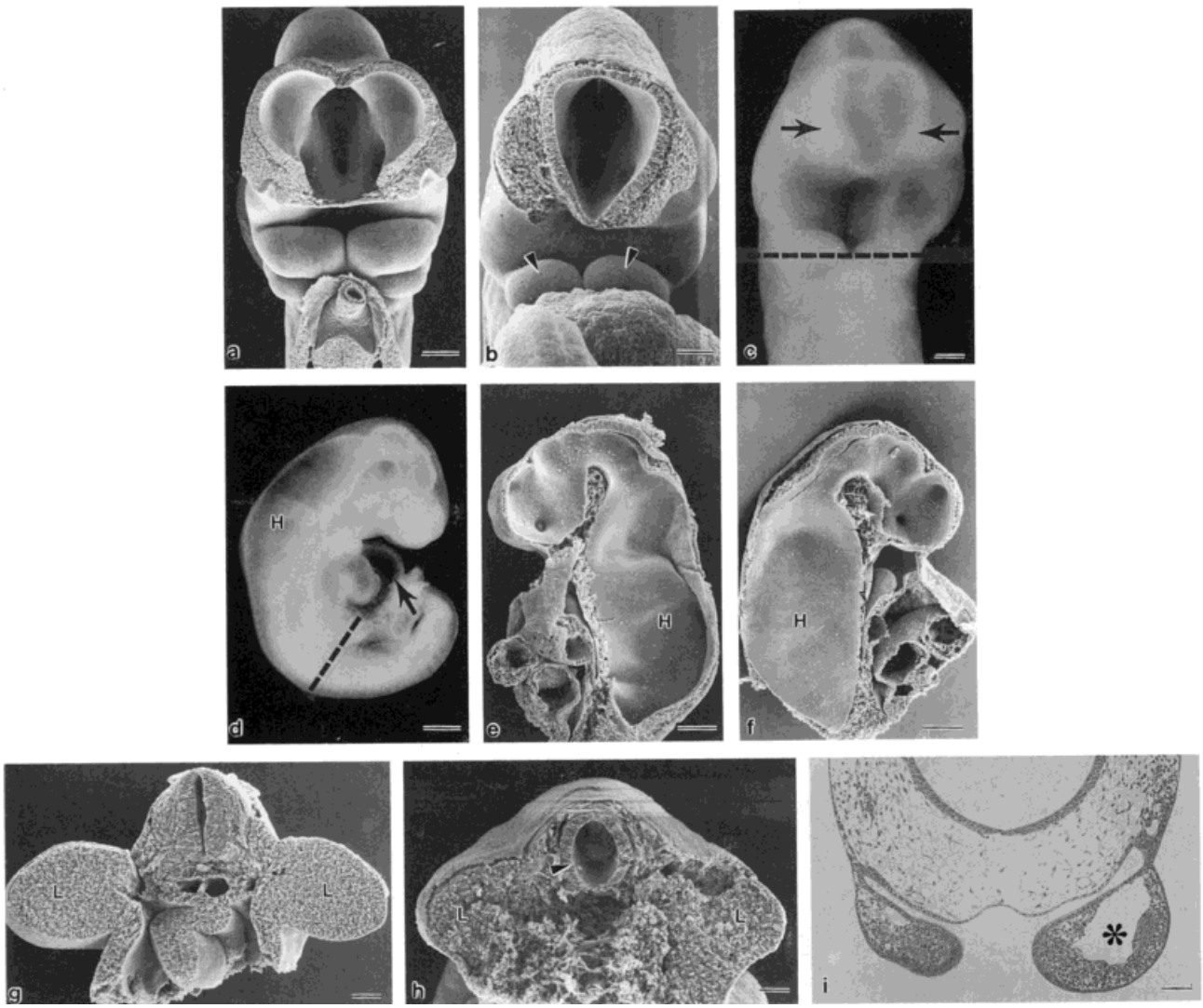

Fig. 3. Scanning electron micrographs and light micrographs of affected (**b–f,h,i**) GD 12 rat embryos following maternal BM 15.766 treatment on GD 6–9 illustrate typical dysmorphogenesis, as compared to comparably staged control specimens (**a,g**). Illustrated in a–c are frontal views, with frontal cuts made through the forebrain in a and b; d is a lateral view; e and f are reciprocal halves of an affected embryo cut in the sagittal plane, and g–i are viewed at the levels indicated by the dotted lines in d (for g and h) and c (for i). Notable are narrowing of the forebrain (arrows) and reduction in the size of the mandibular prominences of the first pharyngeal arches (arrowheads) in b and c; distension of the hindbrain (H) and pericardial cavity (arrows) in d–f; distension of the developing spinal cord (arrowhead) with concurrent thinning of the neuroepithelium and reduced size of the upper limb buds (L) in h as compared to the control specimen in g; and the abnormally large vascular space in the mandibular prominence of an affected embryo as indicated by the asterisk in i. Bars = 200 μm (a,d,e,f); 100 μm (b,c,g,i); and 50 μm (h).

teratogenically diminished cholesterol levels that were present at early gastrulation stages (GD 9–10 in rats), and holoprosencephaly was induced following both treatment times. The later exposure time resulted in more embryos with hindbrain distention. It is expected that this is representative of edema resulting from initial insult either to the yolk sac or the developing heart. Although further analyses are required to examine the effect of altered cholesterol biosynthesis on these tissues, it is noteworthy that Farese et al. [1996] have recently suggested that yolk sac synthesis could serve as an important source of cholesterol.

Fig. 4. Scanning electron micrographs and light micrographs of control (**a,c,e,g**) and affected (**b,d,f,h**) GD11 rat embryos illustrate the presence of abnormal cell populations in the latter group. Illustrated in a and b are frontal views of the anterior neural folds prior to fusion; c and d are dorsal views of the midbrain and hindbrain neural folds; e and f are high magnification views of the boxed areas in c and d, and g and h are histological sections made at the level indicated by the dotted line in c. Abnormal-appearing cells are located at the rim of the anterior neural plate in the region of the forebrain (arrowheads in b) and lower midbrain and hindbrain (arrowheads in f and h). Bars = 50 μm (a–d); and 10 μm (e–h).

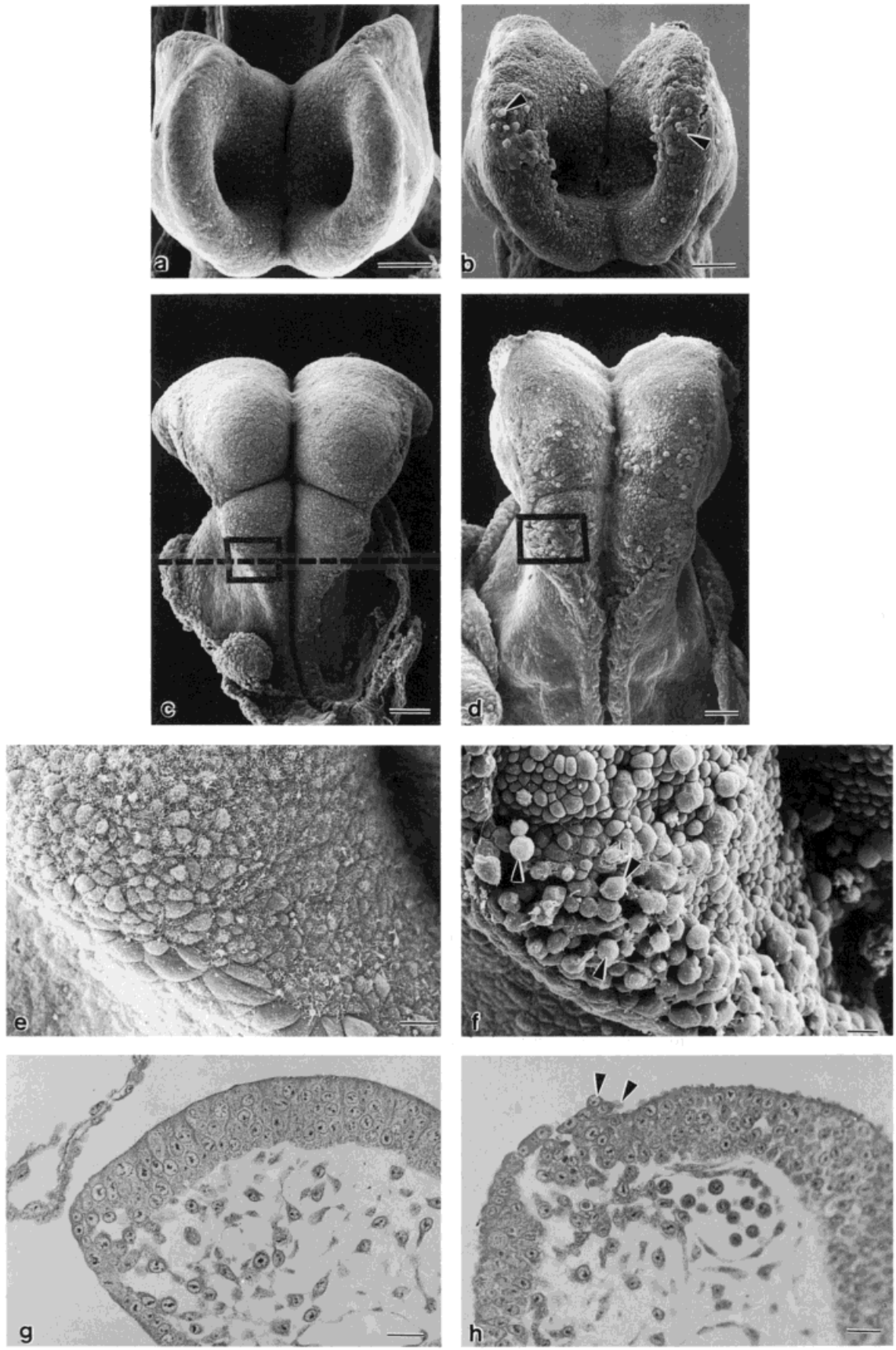

Fig. 4.

The anomalies present in SLOS children that are also present in this model include forebrain abnormalities that fall within the realm of holprosencephaly. Hydrocephaly and hindbrain defects including absence of the cerebellar vermis are also noted in SLOS children. It is expected that at later developmental stages, the narrow cerebral aqueduct noted in our model would lead to internal hydrocephaly. In fact, as early as 1964, Roux noted that hydrocephalus results from inhibition of cholesterol biosynthesis in rats. It is also expected that the lower midbrain and upper hindbrain defects noted in our model would later present as cerebellar defects, in particular involving the dorsal midline area, i.e., the vermis. Future studies will be directed toward examining affected pups at later developmental stages.

Although the model described herein differs from human SLOS by virtue of the fact that maternal cholesterol biosynthesis is a major target in the rats, as opposed to an inherent genetic defect in the affected individuals, many of the resulting major malformations appear to be comparable. This may reflect the possibility that the drug, in addition to affecting maternal cholesterol biosynthesis, also directly targets this biosynthetic pathway in the embryo itself. Little is known regarding the ability of the embryo, at the stages of interest for the current investigation, to synthesize cholesterol. Although embryonic tissues in the rat express high levels of HMG CoA reductase (the rate-limiting enzyme of cholesterol biosynthesis) as early as GD 7–12 [Brewer et al., 1993], it is not clear whether this reflects a requirement for cholesterol biosynthesis or for mevalonate-derived farnesylated products. In favor of the former are data from Pratt [1982] who has demonstrated that mevalonate is converted to lanosterol (implying cholesterol synthesis rather than synthesis of other mevalonate-derived products) in preimplantation-stage embryos. This investigator has concluded that it is likely that the embryo assembles most of its membrane cholesterol de novo. In addition, data from an investigation of later staged rat embryos that utilized incorporation of tritiated water into cholesterol to determine the sources of cholesterol during fetal development, showed extensive endogenous cholesterol synthesis on GD 14. In this study, a high maternal cholesterol diet suppressed maternal cholesterol synthesis but did not reduce the amount of labeled cholesterol in the embryos, thus confirming the endogenous source of newly synthesized cholesterol [Belnap and Dietschy, 1988]. Further investigations are required to determine the capacity of embryos at the time of insult for the current investigation, i.e., GD 10–12, or corresponding to the third and fourth weeks of human gestation to synthesize cholesterol.

In conclusion, the results of this study illustrate the sensitivity of embryos at early stages of embryogenesis to teratogenesis resulting from altered cholesterol biosynthesis. The described drug-induced malformations appear to parallel many of those comprising a human genetic syndrome (SLOS) that also results from altered cholesterol biosynthesis, thus suggesting that this genetic abnormality has a major impact on development early in human gestation. This study has also resulted in identification of cell populations that are particularly sensitive to these alterations in cholesterol biosynthesis and has set the stage for additional studies directed toward understanding the basis for this selective vulnerability.

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